GROSS AND MICROSCOPIC LESIONS IN CHICKS INOCULATED WITH A FILTRATE OF AN AVIAN VISCERAL LEUKOSIS-LIKE AGENT

by

JANICE M. LILLY

B. S., Kansas State University, 1960
D. V. M., Kansas State University, 1962

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1963

Approved by:

Major Professor

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INTRODUCTION

A study of avian visceral leukosis, or visceral lymphomatosis, assumes major importance when considered from either of two viewpoints. First, the condition is one of the most economically devastating diseases confronting the poultry industry. In the United States alone, annual losses due to visceral and neural lymphomatosis have been estimated at 73 million dollars with the visceral form responsible for two-thirds to three-fourths of the mortalities (United States Department of Agriculture, 1955). Secondly, because of its essentially neoplastic nature, fowl lymphomatosis is of particular interest to research workers investigating the etiology of cancer. It has been stated that the visceral form is truly cancerous, caused by a virus-like agent (Burmester, 1952), and is the only avian malignancy known to be contagious in nature (Beard, 1957).

Experimental procedures have repeatedly confirmed that the disease can be reproduced by viral filtrates of lymphoid tumors (Burmester, 1961). These reports include investigations of several different tumor strains originally obtained from visceral lymphomatosis field cases (Burmester et al., 1946; Burmester and Cottral, 1947; Burmester, 1947; Burmester and Denington, 1947; and Burmester and Gentry, 1956).

Observations in this study concern the filtrable agent of an extremely virulent transmissible tumor isolated from a Kansas turkey flock. The objects of this investigation were to determine the outstanding gross and microscopic pathology produced by the

leukosis-like agent following its inoculation into young chicks, and to provide information which would characterize the agent in respect to its mortality rate and mean death time.

REVIEW OF LITERATURE

Nature of the Disease

The three general types of leukosis in poultry were first recognized by Ellermann (1921) and were designated on the basis of involvement of the three main blood cell types as erythroid, myeloid and lymphoid. The erythroid and myeloid types were considered leukemic, but the lymphoid type was generally accepted to be aleukemic and referred to as visceral lymphomatosis or lymphocytoma.

De Ome (1940) discussed the nature of avian lymphomatosis and stated that it was an extravascular, aleukemic, neoplastic condition of the undifferentiated perivascular tissues capable of producing cells of the lymphoid type. It was his opinion that the condition exhibited the four characteristics of neoplastic growth, (1) progressive and uncontrolled proliferation of cells; (2) metastasis; (3) invasion, displacement, and replacement of normal tissues; and (4) predilection for certain tissues and locations.

In a discussion of fowl lymphomatosis as a disease complex, Burmester (1952) recognized three forms, (1) neural; (2) ocular; and (3) visceral. Lymphocytoma, lymphosarcoma and lymphoblastoma were listed as additional terms applied to the visceral type.

Though the condition known as osteopetrosis often occurs in birds having visceral lymphomatosis, Burmester did not consider it as an expression of that disease, since the principal lesion was not lymphocytic in character, but rather an extensive exostosis.

Burmester (1960) also discussed the use of the term visceral lymphomatosis by English workers to describe tumors of the viscera, particularly the gonads, which resembled lesions of the nerve and iris in neural and ocular lymphomatosis. Such tumors were thought to be a manifestation of inflammatory response rather than a neoplastic condition and were considered a visceral form of neural lymphomatosis or fowl paralysis. Campbell (1956), one of the British workers preferring this classification, regarded lymphoid tumors showing anaplasia as lymphoid leukosis. An English colleague, Gordon (1960), reported that the latter condition was the most common form of the leucoses and that it could occur as a diffuse tumor growth (big liver disease) or as a discrete nodular tumorous condition.

There have been other reports than those from England which associate neural and visceral lesions as expressions of the same disease. After reviewing these papers and conflicting evidence, Gross (1957) stated that indication of distinct and separate etiologic agents for the two forms was supported by historical-epidemiologic considerations, the age variation in susceptibility to neural lymphomatosis, the difference in distribution of lesions, their histopathologic character, and transmission studies with cell suspensions. He concluded, therefore, that the etiologic agent of avian visceral lymphomatosis has "certain characteristics

not shared by those of other related forms which are distinct pathologic entities".

Robinson (1958) presented a review of the literature concerning avian lymphomatosis, including information on history, characteristics and properties of the virus, immunological research and transmission studies. The reader is referred to that work for a complete discussion of these aspects of the disease.

Naturally Occurring Cases

Pappenheimer (1929), in an examination of 60 paralyzed birds, discovered that six exhibited visceral tumors and he assumed these were a manifestation of the agent causing paralysis. A histological study of visceral lesions showed them to consist of closely packed, small, round cells with deeply stained nucleus and scant cytoplasm. In some areas exhibiting active proliferation with numerous mitotic figures, cells were larger, nuclei vesicular with a distinct chromatin structure, and cytoplasm more abundant. Pappenheimer concluded such cells might be favorably compared to larger lymphoid elements observed in the centers of germinal follicles. The parenchymal tissue of affected organs was invaded and the elements pushed apart by these cells. Little or no degeneration of organ parenchyma was apparent but the latter was eventually replaced by tumor tissue. Necrosis did occur in some larger tumor masses, but in such areas a broad collar of living cells usually remained around blood vessels.

In one of the first reports concerning transmission of visceral lymphomatosis, Pentimalli (1941) described lesions observed

in a naturally occurring case selected as the donor for attempted transmission. Grossly, the bird presented an enlarged liver which was covered with grayish-white spots separated by hepatic tissue of normal color, a spleen of uniform reddish-brown color, and ovaries exhibiting small whitish nodules. Microscopically, the normal hepatic tissue was reduced to narrow bands separating neoplastic nodules. The latter consisted of loose connective tissue stroma containing large, round, tumor cells with chromatin-rich nuclei, nucleoli, and a variable amount of cytoplasm. Mitoses were frequently observed. Hepatic arteries were intact, but veins frequently showed destruction of the walls with neoplastic tissue occasionally present in the lumina. In some areas, small periportal veins were obliterated by compression. Few blood vessels were seen in the neoplastic tissue, but necrosis was not observed. Normal structure of the spleen was so altered that no remnants of follicles could be distinguished and venous sinuses were barely visible. Though no gross lesions had been noted in the kidneys, histological examination revealed a condition identical to that in the liver. Renal parenchyma was destroyed by compression and tumor cells had infiltrated between renal tubules. Ovarian nodules which had been seen grossly were also composed of tumor cells.

In the same year, Olson (1941) similarly reported lesions of a field case which he attempted to transmit to chicks. He described the liver as moderately enlarged with a number of gray-white tumor masses which were generally discrete, though some tended to be confluent. The spleen was of normal size and color but possessed four small foci similar to those seen in the liver.

Immediately dorsal to the cloaca was a solid round mass of soft, gray tissue about 4.5 cm in diameter which apparently arose from the wall of the bursa of Fabricus. Cells of the tumor masses, as seen microscopically, were large, round, lymphoid cells with relatively large, vesicular nuclei and basophilic cytoplasm confined to a narrow rim at one edge. Mitotic figures were numerous. In general, the neoplastic cells were extravascular in location, though in some organs, for example the liver, cells were observed occasionally within the vessels. In the liver, tumor masses were diffuse and tended to compress hepatic tissue rather than infiltrate it, and multiplying tumor cells appeared in many periportal areas. A generalized hyperplasia of reticulum in the splenic pulp and several microscopic foci of neoplastic lymphoid cells characterized the lesions of that organ. The tumor in the wall of the bursa of Fabricus was composed of the malignant cells and the ovary, though showing no gross abnormality, was also infiltrated by them.

Variation in transmissibility of naturally occurring cases was tested by Burmester and Denington (1947), who chose donor birds on the basis of massive tumefaction of liver or ovary. Of the ten naturally occurring cases selected, all had a lymphomatous liver and spleen and all but one exhibited similar involvement of kidney and ovary. Other tissues grossly or microscopically involved were: bone marrow, pancreas, thymus, sciatic nerve, brachial plexus, adrenal gland, heart, intestine, skin, proventriculus, bursa of Fabricus, mesentery, muscle, pericardium and eye. The liver of one bird was diffusely tumorous, had a smooth surface,

and was extremely friable. The others showed either miliary or focal tumors of varying size, exhibited a granular or irregular surface, were firm and resistant to sectioning. All ovarian tumors were classified as diffuse, since they were consistent lymphomatous masses with very little stroma. Microscopic study revealed tumors to be composed mainly of lymphocytes and larger immature cells which appeared to be hemocytoblasts. Cells were almost entirely extravascular and extrasinusoidal in hepatic tissue.

A survey made on condemned carcasses from a poultry processing plant by Benton and Cover (1957) revealed excessive condemnations due to visceral lymphomatosis with an unusually large number of muscular lesions involved. Grossly, the liver, spleen, and ovaries were most consistently affected, though nearly every organ was eventually observed to be involved. Livers were typically enlarged with diffuse or nodular type lesions. In some cases of diffuse type the liver had a gelatinous consistency and was exceedingly friable. The spleen in some cases was enlarged to three or four times its normal size and had a milky-white appearance. Affected ovaries appeared hyperplastic, granular and enlarged. Histologically, the lesions were characterized by extensive infiltration of both mature and immature lymphocytic cells with the mature type predominating. Cell infiltration was also seen in tissues which had not been affected grossly. Skin lesions were generally associated with the feather follicle and in extreme cases formed scabs with a brownish crust formation.

More typically, lesions were whitish distinct nodules and were most easily detected in the dressed bird. Microscopically the lesions consisted of lymphocytic cells accumulated primarily in the dermis and arranged as circular foci. Muscular alterations were seen in both deep and superficial muscle layers, the most frequently involved group being the pectoral muscles. Lesions varied in severity from narrow, whitish streaks to nodular formations which were elevated above the surface and produced tissue necrosis. Some tumors caused tissue distortion by producing a "ridging effect", these areas being whitish-gray to yellow-orange in color. The most severe cases exhibited necrosis with ulcer formation, accompanied by a gelatinous exudate. Muscular changes were noted to occur in the presence or absence of visceral lesions and were also seen on well-fleshed birds which showed no evidence of systemic involvement. Microscopic lesions varied in severity corresponding to the degree of pathology observed on gross examination. Slight lymphocytic infiltration between fibers with little change in the muscle tissue itself was characteristic of the least affected muscles. More advanced cases showed lymphocytes separating, destroying, and eventually replacing muscle fibers. Degenerating fibers were fragmented and exhibited loss of myofibrils.

Two similar reports of visceral lymphomatosis in turkeys have recently appeared in the literature. Simpson et al. (1957) described the changes seen in a flock of 1200 which had experienced a loss of 60 birds due to the disease. Gross tumors were

most consistent in spleen, liver, and kidney. Spleens were markedly enlarged, the capsules thickened, and white foci appeared in the parenchyma. Livers were also enlarged and possessed multiple elevated areas on the surface which were white, firm, and well-defined. These areas extended to the interior and frequently little normal appearing hepatic tissue remained. In one bird, neoplastic growth had resulted in rupture of the liver capsule with subsequent fatal internal hemorrhage. Affected kidneys were swollen, with tumorous growths which were white, firm, and diffuse. Ovaries were frequently involved with tumors and in three males a large, firm, white, round to oval growth was found intimately attached to the testicle. Heart involvement was characterized by the presence of white nodules or pale lines. The pancreas was affected in many birds, appearing swollen and presenting a nodular surface. Portions of intestinal wall were thickened by white, homogeneous masses, the terminal part of ileum and ceca showing annular enlargement so that the lumina were almost occluded. The duodenum was grossly normal but lesions were detected microscopically. Infrequently, small, white nodules were noted in the proventriculus. A study of the histopathology involved showed all lesions to be composed of dense masses of lymphoid cells which had infiltrated and replaced normal tissue. The predominant cell was lymphoblast-like, possessing a scant amount of cytoplasm and a large, rounded, basophilic nucleus with a distinct nucleolus. Nuclear chromatin was primarily peripheral and several cells showed mitotic figures. In the liver, islands and strands of

hepatic cells which had undergone albuminous or fatty degeneration persisted between the masses of lymphoid cells. Accumulations of the cells commonly invaded the adventitia and media of portal vessels, and in many areas of affected portal triads, only vague silhouettes of bile ducts remained. In the kidneys foci of lymphoblast-like cells had destroyed tubular elements, infiltrated glomeruli and interstitial tissue, and caused albuminous or hydropic degeneration of tubular epithelium. Affected myocardial tissue showed muscle fibers to be separated and broken by focal and diffuse accumulations of the tumor cells. In the pancreas, tumor cells had primarily infiltrated the interstitial tissue causing separation, degeneration, and destruction of glandular elements; islands of Langerhans were rare in tumor-invaded glands. Tumorous intestinal tissue showed involvement of the entire wall, from outer muscle layer to mucosa. In duodenal sections, the most prominent lesion was in the lamina propria, which was greatly thickened. Mucosal glands were compressed or eliminated, and the surface epithelium ulcerated, but outer muscle layers were only slightly affected. The terminal ileum was more often infiltrated by tumor cells than other portions of the intestinal tract, and in such areas the entire wall from outer muscle layer to mucosa was a mass of lymphoid tissue containing a few isolated muscle strands. As in the duodenum, surface epithelium was ulcerated and mucosal glands nearly obliterated. Cecal tumors were similar to those in the duodenum and terminal ileum, but also showed mild separation and disruption of muscularis mucosa and occasional involvement of the inner layer of tunica muscularis. The

outstanding pathology of the proventriculus was a thickening of the lamina propria due to lymphoid cell infiltration. Some destruction of superficial glands was apparent but superficial epithelium and deep glands were unaffected.

Another review of a visceral lymphomatosis outbreak in turkeys was presented by Newberne and Vosbrink (1958). They found the spleen enlarged in every bird, with the capsule invariably thickened, and the parenchyma studded with minute gray areas occasionally interrupted by hemorrhagic foci. Livers were typically enlarged with a diffuse grayish background contrasted by red congested vessels, giving the organ a marblized appearance similar to that of diffuse lymphomatosis in the chicken. Affected kidneys were enlarged and presented a diffuse grayish appearance. The number of lobes affected varied from one to three and enlargements were generally bilateral. No lesions were observed in the ovaries, but the testicle of one tom was enlarged and contained a firm bulging mass at the anterior pole. The lung and pancreas presented small grayish-white nodules and thickening of the intestinal wall as long irregular masses was noted. Microscopically lesions of the liver, kidney, and pancreas were nearly identical to those described by Simpson et al. (1957), but other organs showed some minor differences. It was observed that in the intestine mucosal surfaces were necrotic in some cases, but more typically were thickened and demonstrated greatly increased mucous cell activity. Spleens consistently exhibited hemorrhage and focal infiltration of the malignant cells. In some areas masses of lymphoid tissue replaced from one-third to one-half of

the splenic tissue. Lung lesions were characterized by large masses of the tumor cells obliterating large areas, frequently invading larger air passages, and some pulmonary vessels. In the myocardium, cellular infiltration was sufficient to cause separation of fibers but no nodular foci were observed, which accounted for the lack of gross lesions in the heart. The mass which was observed attached to a testicle was also composed of tumor cells, accompanied by a few connective tissue fibers. A thick connective tissue capsule surrounded the mass which appeared to have originated as an extension of testicular neoplasia following rupture through the organ tunic.

An acute form of visceral lymphomatosis causing high mortality in young chickens was described by Frederickson and Burmester (1961), who reported that outbreaks had been observed in 11 flocks of six Eastern Seaboard states. The fulminating course of the disease was indicated by the well-fleshed appearance of dead birds. Tumors usually involved several organs in the same bird and were seen in the following organs with decreasing frequency: ovary, liver, kidney, spleen, lungs, bone marrow, heart, intestine, muscles, nerves, and subcutis. The predominant lesions were large, soft, gray tumors but in some cases swollen, congested livers and spleens more closely resembled erythroblastosis than visceral lymphomatosis. The tumors were judged to be very malignant on the basis of histological studies, as component cells were large, anaplastic, and lymphoid in character, with many mitotic figures present. The authors stated that history of the

outbreaks gave few clues as to source of infection or mode of transmission for the tumor viruses concerned.

Transmission by Cellular Inocula

Gibbs (1936) reported that his early attempts to transmit lympholeukosis (visceral lymphomatosis) from diseased to healthy birds resulted in failure, possibly, he surmised, due to destruction of cells in the preparation of inocula. In his examination of field cases he had noted that large numbers of lymphoblasts appeared in portal blood, some of them in mitosis. He removed blood from five such cases and inoculated 25 chickens, of which 12 contracted the disease. On necropsy he found lesions were comparable to those of field cases. Anemia, emaciation, and liver enlargement were the changes cited. Gibbs concluded that the causative agent of lympholeukosis was associated with the lymphoblastic cells observed in the blood and that cells of the same type were found in the liver, spleen, and portal blood of diseased chickens.

The second and third reports of successful visceral lymphomatosis transmission appeared in the literature in the same year, 1941. These experiments were conducted separately by Pentimalli (1941) and Olson (1941), and the lesions they observed in their donor birds have been described previously in this review. Pentimalli attempted to transmit the disease by intramuscular implants. He observed a growth of tumors at the inoculation site and carried the neoplasm through 23 transplant generations within a year, consistently reproducing the characteristics

of the primary lesion. In general, tumors reached the size of walnuts in one to two weeks and then infiltrated the muscle killing recipient birds in four to eight weeks. Few lesions were noted in the internal organs of implanted birds. Of 92 birds which developed tumors, secondary neoplastic growths were seen in liver and kidneys three times, lungs and heart in two cases, and only once in splenic tissue. It was stated, however, that histological examination of organs would probably have revealed more evidence of metastasis.

Olson (1941) also accomplished transmission by intramuscular implants of tumor tissue, and 30 serial passages were completed. Of 442 chickens inoculated, growth of the implants was observed in 67.7 per cent and of these 17.0 per cent demonstrated metastasis to internal organs. Diffuse metastasis was more frequently noted in later passages and in such cases birds usually died nine to 12 days after inoculation. The sites of predilection for secondary tumor growth were given in decreasing order of frequency as follows: heart and proventriculus, adrenals, kidneys, gonads, liver, spleen, thymus, mesentery, lung, and thyroid. Lesions of the heart, liver, and kidney were usually circumscribed and seen grossly, while in the spleen foci of tumor cells were often detected only upon microscopic examination. The adrenals, thymus, gonads, bone marrow, mesentery, and wall of the proventriculus were described as being diffusely infiltrated with neoplastic lymphoid cells. The cell type observed in the donor bird retained its general characteristics through the serial passages. Outstanding features of the cell as cited by Olson were the

vesicular nature of the nucleus, margination of nuclear chromatin, a relatively large and distinct nucleolus, and a markedly basophilic cytoplasm.

Three years after his initial report, Olson (1944) presented additional information obtained by carrying the tumor strain to its 132nd serial passage. It was noted that cases characterized by tumor growth with diffuse metastasis were the most violent and fulminating conditions seen and that the visceral changes usually consisted of notable enlargement of liver, spleen, and occasionally kidneys. The liver was generally red-brown in color and histologically infiltrated by neoplastic lymphoid cells. These appeared to occupy an extravascular position beneath the reticuloendothelial lining of sinusoids, though some cells were found within them. The tumor cells tended to proliferate in some localized areas around blood vessels, but not always in direct connection with periportal areas. Hepatic cells were crowded and distorted and many were in various stages of degeneration. Spleens were usually purple-red in color, possessed a tense capsule, and were filled with neoplastic lymphoid cells when examined microscopically. Tumor cells were often found in the vascular bed of other organs, including kidney, lung, heart, and thymus.

Burmester and Prickett (1945) were able to develop four strains of highly malignant tumors from naturally occurring lymphomatosis cases (RPL Strains 14, 15, 16, and 17). Intramuscular injection of cells from all strains produced tumors at the injection site in seven to 14 days, few birds dying without showing

gross lesions in one or more of the viscera. Visceral involvement was assumed to be due to metastasis. The organ most often altered was the liver, which was frequently enlarged to three times normal size and presented diffuse tumor areas of the miliary type. Besides the liver, other organs most affected were kidney, gonad, spleen, and serosa. The kidneys, spleen, and ovary were diffusely involved, whereas heart lesions generally appeared as separate nodules. Organs showing either focal or diffuse involvement were testes, lungs, mesentery and peritoneum. Microscopic lesions of typical cases from each of the four strains showed definite similarities. The predominant cellular element was an immature lymphoid cell with basophilic cytoplasm and large nucleolus. After comparing the RPL strains with those studied by Pentimalli (1941), Olson (1941), and Brewer and Brownstein (1946), Burmester and Prickett concluded that with the same route of inoculation, pathological changes and transmission characteristics of all the strains were similar. Further, they stated that results of these studies proved tumors from cases of naturally occurring visceral lymphomatosis could give rise to highly malignant transplantable strains. These propagated strains, however, appeared in all respects to demonstrate a much greater malignancy than the disease in its natural form.

The experiments conducted by Brewer and Brownstein (1946) gave indication that an infective agent was involved in the successful transmissions. While results of the previous workers could have been explained by transplantation of malignant cells, these men noted that chicks brooded in contact with inoculated

birds suffered significantly higher mortality from lymphomatosis than did controls brooded in other pens. From this observation they reasoned that inoculated chicks must have carried unusually large amounts of the etiological agent of the disease in order to have increased its incidence among contact controls. They gave only a brief discussion of gross and microscopic lesions, but Burmester and Prickett (1945) reported this strain to be similar pathologically to the RPL Strain 17 described in their 1945 publication.

Following inoculation of cell-containing material from a lymphomatous field case, Davis and Doyle (1947) reported an increased incidence of visceral lymphomatosis and a shortened incubation period among inoculates as compared to controls. They also reported that inoculation of various tissues from donors suggested no marked concentration of the causative agent in any certain organ or organs. Two years later these workers presented results of serial passages of their strain (Davis and Doyle, 1949). They found lymphomatous lesions in 94 per cent of the livers examined, 89 per cent of the ovaries, 79 per cent of the hearts, 73 per cent of the kidneys, and 69 per cent of the spleens. Upon repeating the trials of various organs as donor tissue, they found the liver to cause a significantly higher percentage of deaths while ovarian tissues resulted in the fewest successful transmissions.

Davis (1952), after carrying the RPL Strain 16 through 30 serial passages with cellular inocula reported that 96 per cent of the birds showed tumorous livers. In a second series of 15

succesive passages, 92 per cent exhibited liver involvement. Spleens and kidneys also frequently showed gross lesions. Gonads, heart, pancreas, proventriculus, gizzard, mesentery, and adrenals were less often affected.

Also using the RPL Strain 16, Eyestone (1953) discussed pathogenesis of the disease as it occurred following intramuscular inoculation. Grossly, he observed tumor development and necrosis at the inoculation site, enlargement of the proventriculus, and slight enlargement, congestion, and some hemorrhage in the liver. Microscopically, there was an increase in the amount of lymphoid tissue around portal vessels of the liver, and terminally an invasion of sinusoids by tumor cells. Extensive hemorrhage and areas of necrosis were present in the parenchyma. Metastatic foci of tumor cells also appeared in the kidney with subsequent diffuse infiltration of cells between cortical tubules. The proventriculus exhibited large numbers of tumor cells in submucosal glandular areas with some occasionally appearing near the tips of villi. In the lungs and spleen, Eyestone described proliferation of some lymphoid nodules, with infrequent tumor cells in blood vessels during terminal stages of the disease. Other tissues examined but which exhibited no alterations were pancreas, intestine, adrenal gland, thymus, and heart. On the basis of his histological studies, Eyestone concluded that following inoculation of RPL Strain 16, the donor cells were propagated at the inoculation site and grew until death resulted from metastases. He assumed there was no transformation of existing lymphoid tissues to neoplastic cells.

Transmission with Cell-Free Preparations

The first reported transmission of visceral lymphomatosis by inoculation of cell-free material appeared in the literature in 1933. The data presented (Furth, 1933) were the result of inoculation of 73 chickens with cell-free filtrate from a visceral lymphomatosis field case. Among the injected birds Furth observed 23 cases of leukosis, 11 of which were designated as lymphomatosis. In addition, three birds developed myelocytomatosis, and one endothelioma was noted. The author considered the rarity of the latter two neoplastic conditions to suggest the probability that they were caused by the same agent responsible for the leukosis cases.

Considerable time elapsed before the next successful filtrate experiment was reported. Burmester et al. (1946) obtained the tumor strain developed by Olson (1941), and referring to it as RPL Strain 12 stated that it possessed a filtrable agent capable of producing lymphoid tumors and osteopetrosis in chickens. Inocula which contained viable tumor cells induced tumor growth at the inoculation site with resultant metastasis to viscera and death of all birds in an average of 10.2 days. Plasma filtrates injected by various routes into two or three day-old chicks induced a high incidence (86 per cent) of lymphomatous tumors of the viscera, with many birds also developing osteopetrosis. No tumors appeared at inoculation sites and the incubation period, two to six months, was much longer than in cellular inoculation experiments. The lymphomatosis cases which developed ranged from

acute to chronic in form. In the acute type organs were usually diffusely involved, the liver being friable and enlarged to twice normal size. Livers of birds exhibiting more chronic lesions had developed nodular or focal tumor growths often leaving little visible liver parenchyma. Such tumorous livers were firm, friable, and often enlarged as much as four times normal. Other viscera were also occasionally affected. A summary of the gross involvement of viscera in 45 filtrate inoculates was given as follows: liver--93 per cent; spleen--82 per cent; kidney--58 per cent; gonad--31 per cent; heart--22 per cent; serosa--13 per cent; pancreas--9 per cent; proventriculus--9 per cent; adrenal--4 per cent; and intestine--4 per cent. The histological changes were observed to be similar to those described by Olson (1941), Burmester and Prickett (1945), and Jungherr (1948). There was, however, evidence of a more localized type of leukemoid reaction in the viscera and focal areas of necrosis appeared in the liver, the latter being attributed to infarctive processes related to tumor growth. Burmester contended that his data were not sufficient to enable him to state whether or not the osteopetrosis and visceral tumors were due to a single entity or resulted from more than one agent.

One year later Burmester and Cottral (1947) published the results of six serial passages of the RPL Strain 12 filtrable agent. The total tumor incidence observed in all groups was 81 per cent and the average days-to-death was 137. Filtered plasma of tumor-bearing birds produced about as high an incidence of tumors as did filtrates of lymphomatous livers, and, after the

first passage, filtrates appeared to be as effective in producing the visceral tumors as were cellular suspensions. Gross lesions of the viscera observed in 127 cases were in the following proportions: liver--99 per cent; spleen--79 per cent; kidney--58 per cent; gonad--22 per cent; heart--9 per cent; proventriculus--3 per cent; and peritoneum--2 per cent. Again, a high incidence of osteopetrosis was observed in filtrate inoculates. The authors also noted that the intraperitoneal route appeared to be about as effective as the intravenous route.

To further demonstrate the transmissibility of the disease with cell-free materials, Burmester (1947) repeated his earlier experiments, but RPL Strains 18, 19, 20, and 21 were the tumor types tested. Both tumor filtrates and plasma from affected birds produced a high incidence of visceral tumors in recipients. Cases of osteopetrosis also developed in some of the birds inoculated with Strains 18 and 21. The main pathological characteristic of all strains was a lymphomatous involvement of many visceral organs. The liver was most often tumorous, followed closely by the spleen, then by the kidney and gonad. Grossly, the parenchymatous organs appeared to be diffusely lesioned, but microscopic study revealed that most tumors consisted of small focal areas. Varying amounts of necrosis with subsequent fibrosis appeared in tumorous livers, Strain 20 most frequently producing this feature. Strains 18, 20, and 21 gave tumor involvement which was primarily extravascular though a few tumor cells appeared in sinusoids and veins. The cell types observed were lymphocytes, lymphoblasts, hemocytoblasts, and intermediate

forms. Another distinctive characteristic of Strain 19 was its relatively short incubation period, 21 to 43 days in later passages. Burmester noted that intravascular tumor tissue and a short "latent" period were also seen in the easily transmissible erythrogranuloblastosis. He suggested that this might indicate at least one of the agents of Strain 19 was similar to the transmissible agent of other types of leukosis previously described by several investigators.

Burmester and Denington (1947) studied the variation in transmissibility of naturally occurring cases of visceral lymphomatosis with both cellular inocula and cell-free preparations. Of 10 donors tested, tissues from four yielded infective filtrates, and these produced visceral lesions in 39 to 94 per cent of the recipients. The conclusion reached was that some, but not all, cases of visceral lymphomatosis could be transmitted to chicks by inoculation with filtrates. From a survey of filtrate inoculated birds the authors compiled the following list of organs affected and the relative frequency of lesions appearing in each: liver--98 per cent; spleen--83 per cent; kidney--50 per cent; heart--25 per cent; and gonad--10 per cent.

The effect of dosage on development of visceral lesions following inoculation with RPL Strain 12 filtrate was examined by Burmester and Gentry (1956). They found that deaths could occur as early as the 22nd day in high dosage groups with the average days-to-death being 55.6. In lower dosage groups, however, the incubation period averaged 128.2 days. Dosage also affected the rate of transmission. In high dosage lots as many as 90.1 per

cent developed tumors, whereas the incidence resulting from low doses was only 55.5 per cent. Microscopically, it appeared that increasing the filtrate dosage increased the proportion of intravascular cases which developed.

Despite the numerous publications ascertaining the filtrability of the RPL Strain 12, Campbell (1958) questioned these results. He stated that in the hands of British workers (Darcel and Negroni, 1954) RPL 12 did not seem to be associated with a virus, and further, that it was regarded by some as an aberrant form of erythroleukosis rather than a true lymphoid tumor.

Prior to 1959 all references made to the RPL Strain 12 lymphomatosis agent were based on the assumption that there were three principal disease manifestations: (1) extravascular lymphomatosis; (2) intravascular lymphomatosis; and (3) osteopetrosis. At that time, however, Gross et al. (1959) reported extensive comparative histopathologic studies had revealed the entity referred to as intravascular lymphomatosis to be in reality erythroblastosis. In addition, a benign neoplastic condition, hemangiomatosis, was described as a fourth manifestation of the filtrable agent. The authors summarized the essential nature of visceral lymphomatosis as an extravascular neoplasia arising both intramedullarly and extramedullarly in a multicentric manner, and the typical cell type was described as a highly undifferentiated element of the lymphoid series. Liver, spleen, and bone marrow were considered the organs most frequently involved, with kidney lesions appearing only slightly less often. Tumors of the gonads, particularly the ovary, were listed next in order of

frequency, followed by the bursa of Fabricus. Lesions in other tissues were regarded as less common while the central and peripheral nervous system and thymus were designated as rarely tumorous. The malignant cell of the disease was characterized as possessing a vesicular nucleus, due to margination and clumping of chromatin, and a conspicuous acidophilic nucleolus. It was suggested that nucleoli were in some cases so prominent that they simulated typical viral intranuclear inclusions of other diseases. In general appearance the cell was so undifferentiated that it was considered to resemble a proerythroblast, the primary cell of erythroblastosis. However, two differences were noted which would distinguish the diseases. First, immature cells involved in visceral lymphomatosis appeared extravascularly, whereas erythroblastosis was primarily an intravascular abnormality. Secondly, and most important, young erythroblasts were very uniform in appearance while neoplastic cells of visceral lymphomatosis exhibited considerable variation in size and shape.

A conference on the histopathology of material obtained from inoculations with cell-free preparations of RPL Strain 12 was held in 1959 (Burmester et al., 1959). The decisions of conferres agreed with those stated by Gross et al. (1959). In addition, they also designated the term visceral lymphomatosis as a broad one applied to neoplasia of cells in the lymphocytic series which could show variable degrees of malignancy. It was their opinion that the extent of malignancy could be best expressed by use of the term lymphoblastomatosis for tumors of the more immature cell type and lymphocytomatosis for more differentiated forms.

MATERIALS AND METHODS

Origin and Care of Experimental Birds

All chicks used in this study were New Hampshire Reds of mixed sex.[1] These chicks were received when one day of age and immediately upon arrival at the laboratory were placed in an electrically heated brooder. For at least the first 10 days of life chicks were maintained in the heated brooder and at 10 to 14 days of age were transferred to a non-heated brooder or cage. Responsibility for care of all birds was restricted to two laboratory personnel who had no contact with other fowl. Feed in two forms was used.[2] A starter type was fed to those chicks in the heated brooder, and after transferral to a non-heated unit either a starter or grower type was given. Feed and water were offered continuously and watering jars and troughs were washed daily with a detergent and hot water. Litter was removed twice weekly from the dropping trays of brooders and cages, after which they were sprinkled with chlorinated lime.

Source of Agent

The visceral leukosis-like agent employed in these experiments was originally obtained by Twiehaus and Robinson (1957)

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[1] Maries Valley Hatchery, Westphalia, Missouri.

[2] Poultry Farm, Kansas State University.

from a turkey brought to the Kansas State University Poultry Diagnostic Laboratory. The donor bird was one of a large group from a farm on which the incidence of visceral leukosis was extremely high. In the five years since initial passage from the donor turkey, this agent has been passed serially 182 times, utilizing both chickens and turkeys of various breeds. These passages were accomplished by intraperitoneal injection of tissue homogenates obtained from inoculated birds showing typical signs. With few exceptions, hepatic and splenic tissue was used as donor material.

Inoculation and Selection of Donors

Donor chicks were inoculated intraperitoneally at 10 to 20 days of age with 0.1 ml of a liver-spleen suspension prepared from an inoculated bird. This cellular inoculum was obtained by grinding one gm of donor tissue in nine ml Simm's solution (see Appendix) utilizing a Potter-Elvejhem tissue homogenizer. Five or six days after inoculation, chicks to be used as donors were selected. Selection was based entirely on clinical signs of droopiness, empty crop, watery fecal material, and ruffled feathers.

Preparation of Filtrates

All utensils employed in filtrate preparations were either stainless steel or Pyrex glass products which had been sterilized by autoclaving. Prior to commencing an experiment, this equipment was placed in a refrigerator and chilled for a minimum of

30 minutes. Throughout the preparation procedures aseptic conditions were maintained and materials were kept at ice water or refrigeration temperatures.

Four or five birds were selected from the donor group and sacrificed by cervical disarticulation. The ventral aspect of each bird was soaked with 70 per cent alcohol, the skin incised and pulled toward the head to expose the sternum. The sternum was then removed to reveal abdominal and thoracic cavities. Livers and spleens of all donors were excised, placed in a Petri dish, and minced with scissors. Fifteen gm of tissue were weighed and placed in a cork-stoppered 500 ml Erlenmeyer flask containing approximately 80 gm broken glass. As a diluent, 60 ml of Simms solution were added and the flask was then shaken vigorously for five minutes to bring about rupture of cells. After homogenization, the tissue suspension was decanted into two 50 ml screw-cap tubes, except for a small portion which was inoculated into chicks as a control test of donor tissue infectivity. The tubes containing tissue homogenate were placed in a refrigerated centrifuge[3] maintained at 4°C. and centrifuged at 3000 rpm for 10 minutes. Supernatant material was then removed with a pipette and filtered through a #10 Selas candle,[4] the latter suspended in a 500 ml suction flask by a rubber stopper. This flask was attached to a Cenco Hyvac 7 vacuum pump[5] and a maximum

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[3]International Equipment Company, Boston, Massachussetts.

[4]Chicago Apparatus Company, Chicago, Illinois.

[5]Central Scientific Company, Chicago, Illinois.

negative pressure of five pounds applied. The cloudy fluid obtained after passage through the #10 filter was withdrawn from the flask and similarly passed through a #02 Selas Candle.[6] After 30 to 50 ml of filtrate had been collected the material was cultured for sterility and immediately injected into recipient chicks. A sterility check consisted of inoculating the filtrate into proteose-peptone, thioglycolate, thiol, and selenite broths, and culturing on a blood agar plate. After 48 hours incubation at 37°C., the selenite broth was streaked on SS agar, the thioglycolate and proteose-peptone broths on a blood agar plate. A drop of the thiol broth was placed on a slide, Gram-stained, and examined microscopically for organisms. Agar plates were examined for growth after 48 hours incubation.

Care of Equipment

Following preparation of a filtrate, both #10 and #02 Selas candle filters were backflushed for 20 to 30 minutes. This was accomplished by filling the suction flasks with distilled water, and connecting them via rubber tubing to a gravity-flow distilled water system.[7] By tying candles in place within the flasks, pressure exerted by the flow of distilled water was sufficient to remove a greater part of trapped cellular material from filter pores. Following the period of backflushing, both Selas candles were removed to a 56°C. drying oven overnight. The following day,

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[6]Aloe Scientific, Saint Louis 12, Missouri.

[7]Barnstead Still and Sterilizer Company, Boston, Massachussets.

the #02 candle was again set in a suction flask and autoclaved, prior to testing for bacteriological retention. The organism employed in this testing procedure was a strain of Escherichia coli[8] which produced colonies with a typical metallic sheen on EMB agar. A 24-hour culture of this organism in proteose-peptone broth was cultured on an EMB agar plate as a control. Then approximately 30 ml of the culture was passed through the #02 Selas filter under the same negative pressure utilized in preparation of visceral lymphomatosis filtrate. The filtered broth was streaked on a second EMB agar plate, and at the end of 48 hours incubation at 37°C. both control and test plates were examined for colony growth.

Following filtration of the bacterial culture, backflushing and drying procedures were repeated on the #02 candle. Both the #10 and #02 filters were then removed from their rubber stopper supports, placed for eight hours in an electric muffle furnace,[9] and upon cooling were considered ready for reuse.

Glassware, syringes, and needles employed in filtration experiments were cleaned in a standardized manner. Immediately after use, equipment was washed in hot water with a mild detergent, rinsed three times in tap water, then placed in a solution of Non-Ion-Ox[10] and distilled water to soak overnight. The following day, all utensils were rinsed eight times in tap water,

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[8]Bacteriology Department, Kansas State University.

[9]Hevi Duty Electric Company, Milwaukee, Wisconsin.

[10]Aloe Scientific, Saint Louis 12, Missouri.

once in distilled water, finally in deaminized[11] distilled water, then air-dried.

Experimental Procedure

Chicks inoculated with filtrates were between four and eight days of age at the time of injection. Dosages ranging from 0.1 ml to 0.4 ml were given by the intraperitoneal route, employing a 22-guage, three-fourth inch needle and injecting on the right side of the abdominal region.

A total of 10 filtrate passages were conducted involving the injection of 255 birds. In each experiment 20 to 30 chicks were inoculated with filtered material and five or more chicks of the same age were left untreated as contact controls. Each inoculate was banded in the left wing at the time of injection and controls were similarly identified at that time.

As further controls, a total of 35 chicks were inoculated with filtrates prepared from tissues of non-inoculated birds. Two of these normal-cell filtrates were made and in each case the procedures followed were identical with those utilized in preparation of the visceral lymphomatosis filtrates. In addition, two cellular homogenates of normal chicken tissue were inoculated into a total of 15 chicks.

All birds found dead, whether control or inoculate, were subjected to necropsy and the cause of death determined on the basis of gross lesions. When no lesions were apparent, tissues

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[11]Deeminizer. Crystal Research Laboratories, Inc., Hartford, Connecticut.

were subjected to microscopic examination unless extensive post-mortem degeneration had taken place. Some birds in each experiment were sacrificed when the clinical signs appeared (Plate I, Appendix) and samples of tissues presenting gross lesions were selected for histologic examination.

In each trial, birds which survived filtrate inoculation and all controls were retained a minimum of 30 days. The birds were then sacrificed, necropsied, and organs showing gross lesions were collected for histopathological study.

Survey of Affected Tissues

To survey gross and microscopic lesions, 45 filtrate-inoculated chicks showing typical clinical signs (Plate I, Appendix) were sacrificed 10 to 13 days after injection. Immediately after death, each chick was weighed and subjected to a complete necropsy. Gross lesions were recorded, including information on the dimensions, appearance, and tissue affected. The liver, spleen, and thymus lobes were then removed and each organ weighed separately. As controls, 30 untreated chicks of the same age were similarly examined. All weights were obtained on a Right-A-Weigh scale[12] and were recorded to three significant figures.

To fully assess the extent of lesions, major tissues were collected from each chick for microscopic examination. Blood and bone marrow were excluded from the survey as changes occurring in these two tissues were considered to require a separate

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[12] Wm. Ainsworth and Sons, Inc., Denver, Colorado.

and more extensive investigation. Samples of the following tissues were included: liver, one section from each lobe; spleen; pancreas; adrenals, left and right; ovary or left and right testicles; kidney, left anterior and right posterior lobes; lung, left and right; heart; thyroid, left and right; thymus, two lobes; trachea; esophagus; crop; proventriculus; ventriculus; duodenum, descending and ascending loops; intestine, one section just anterior to Meckel's diverticulum and the terminal portion of ileum; both ceca, near the junction with ileum; bursa of Fabricus; mesentery; sciatic nerves, left and right; brain, cerebrum and cerebellum; eyes, left and right; muscle, right breast and left thigh. The same tissues were taken from 15 untreated birds of the same age as control samples.

Histological Techniques

Tissues selected for microscopic study were fixed in 10 per cent buffered formalin for a minimum of 72 hours, after which time they were processed in the Kansas State University Pathology Department laboratory. Sections were cut at five microns, stained with Harris' hematoxylin and eosin, coverslipped, and marked for permanent identification.

RESULTS AND DISCUSSION

Transmission

Successful transmission of the visceral leukosis-like agent by inoculation of chicks with cell-free filtrates is illustrated

in Table 1.

Table 1. Transmission of visceral lymphomatosis with cell-free filtrates.

Experiment	Dose (ml)	Age at inoculation (days)	Number inoculated	Visceral lymphomatosis mortality (per cent)	Days-to-death Range	Days-to-death Average	Experimental period (days)
T_2FB_2	0.1	6	15	73.3	9-13	10.6	47
	0.4	6	15	73.3	8-10	9.0	47
T_2FC_3	0.4	8	30	80.0	8-14	12.1	38
T_2FK_1	0.4	4	25	76.0	9-13	10.2	35
T_2FL_2	0.4	5	30	76.7	10-13	10.7	80
T_2FO_1	0.4	7	30	83.3	8-14	10.7	65
T_2FP_1	0.4	5	30	83.3	8-16	10.6	58
T_2FS_1	0.2	7	10	70.0	10-16	12.0	43
	0.4	7	10	70.0	9-13	10.7	43
T_2FT_1	0.4	6	20	90.0	8-12	9.9	37
T_2FT_2	0.4	4	20	85.0	8-11	9.5	32
T_2FU_2	0.3	4	20	90.0	8-17	10.7	40

In the 10 experiments conducted, mortality rates among inoculates ranged from 70.0 per cent to 90.0 per cent. Of the 255 birds inoculated, 205 (80.4 per cent) died and were found to be grossly or microscopically affected by pathological changes like those described previously as typical of visceral lymphomatosis. The proportion of inoculated chicks infected by the filtrable agent was comparable to results obtained by workers using other visceral lymphomatosis tumor strains. Burmester and Denington (1947) reported successful transmissions in 94.0 per cent of recipient birds with filtrate from one tumor strain. Other strains have produced visceral lymphomatosis at rates ranging from 81.0 per cent (Burmester and Cottral, 1947) to 90.1 per cent

(Burmester and Gentry, 1956).

The unusual property of the agent employed in these studies was its rapid action in causing neoplastic lesions and death of inoculates. The average days from inoculation to death varied from nine to 12.1 in the 10 trials, and the average for all 205 inoculates was 11.1 days. The earliest recorded death due to the filtrable agent occurred eight days following inoculation while the longest period was 17.0 days. When compared to results obtained with previously reported visceral lymphomatosis strains, these figures corresponded more closely to mean death times observed following injection of cellular suspensions than to those seen in filtrate inoculates. For example, Burmester et al. (1946) stated that viable tumor cells produced death in an average 10.2 days, whereas following injections of cell-free inocula the shortest average days-to-death reported was 55.6 days (Burmester and Gentry, 1956).

Among contact controls and chicks inoculated with preparations of normal tissue (cellular and cell-free), no deaths occurred in which pathology typical of that produced by the agent was seen. One contact control did develop neural lymphomatosis, as did one filtrate inoculate which survived the usual incubation period of the disease and was killed 41 days after inoculation. These two cases were considered to be of spontaneous origin.

In all experiments conducted, sterility checks on the filtrates failed to reveal the presence of viable organisms. In addition, passage of Escherichia coli cultures through the #02 Selas filters used in filtrate preparations resulted in complete

bacterial retention. Therefore, it was concluded that successful transmission of the disease with filtrates indicated the agent present was capable of passing through bacteria-retaining filters. Furthermore, maximum pore size of the #02 filter, 0.85 microns, precluded the possibility that transmission was due to transplantation of malignant cells.

Gross Weights of Various Organs in Controls and Inoculates

In serial passages with cellular inocula, it was noted that the size of livers and spleens in inoculates was markedly increased when compared to controls the same age. In contrast, lobes of the thymus in affected birds had undergone marked atrophy. Such observations prompted a gross weight survey of those three organs in filtrate inoculated chicks. Tables 4 and 5 in the appendix contain a complete list of the weights obtained in 30 controls and 45 inoculates, while a summary of results appears in Table 2.

Table 2. Summary of liver, spleen, and thymus weights expressed as per cent body weight.

Organ	Average		Range	
	Controls	Inoculates	Controls	Inoculates
Liver	3.890	7.410	3.160--5.720	5.290--10.200
Spleen	0.124	0.509	0.074--0.195	0.140---1.970
Thymus	0.440	0.174	0.162---0.825	0.051---0.381

Enlargement of livers and spleens and depletion of thymus glands were noted grossly in filtrate inoculates and the gross weight study substantiated these alterations. The average weight

of livers from inoculated chicks, expressed as percent body weight (7.41 per cent), was nearly twice that of controls (3.89 per cent) and the average splenic weight percentage of inoculates (0.509 per cent) represented a four-fold increase over that of non-inoculates (0.124 per cent). Conversely, the average thymus weight of inoculated chicks (0.174 per cent) was less than one-half the figure obtained from examination of controls (0.440 per cent). The necessity of converting organ weights to per cent body weight was due to a marked difference in body weights between the two groups. Controls weighed an average of 121.9 gm, the range being 89.0 to 161.0, while the average weight of inoculates was 78.1 gm with a range of 60.7 to 109.0.

Regarding the liver, spleen, and body weights, similar results were obtained in a study on RPL Strain 16, employing a cellular inoculum (Julian, 1953). Liver and spleen weights of inoculates were significantly higher than those of controls, even though the latter group exhibited a much greater average body weight. Julian proposed that a possible reason for cessation of total body growth was interference with synthetic activity of the liver or vital functions of some other organ. Lack of total body growth concurrent with malignant cell growth was suggested to indicate that tissue substances of the bird itself were being utilized by tumor cells in terminal stages of the disease.

The significance of decreased thymus tissue in inoculated chicks was not ascertained. Olson (1941), in his initial study of visceral lymphomatosis tumor transplants, noted that in chickens which exhibited tumors for two or more weeks prior to death,

atrophy of the thymus was a common feature. Involution was considered the most plausible reason for Olson's observation, since any cachectic disease would cause an accidental involution of the gland (Trautman and Fiebiger, 1952). Such an explanation, however, was not fully applicable to this study. Thymus atrophy was noted to occur within five days after inoculation of cellular material, and affected chicks were not cachectic but rather appeared quite well-fleshed. A search of the literature revealed no other reports of similar thymus changes, but the gland was possibly overlooked in earlier investigations.

Survey of Affected Tissues

The relative frequency of gross lesions observed in various tissues of 45 filtrate-inoculated chicks is given in Table 3.

Table 3. Gross involvement of viscera in 45 filtrate-inoculated chicks.

Organ	Per cent involvement
Liver	100.0
Spleen	86.7
Pancreas	62.2
Lung	44.4
Mesentery	33.3
Gonad	31.1
Intestine	26.7
Proventriculus	15.1
Kidney	2.2

Figures resulting from the study were considered to represent the general distribution of lesions as seen in all affected chicks, with the exception of the spleen. Six (13.3 per cent) of

the chicks examined in the study showed no splenic abnormality, whereas necropsy of birds which died of the disease rarely revealed that organ to be completely normal. Since the study summarized by Table 3 involved only sacrificed chicks, the possibility remained that spleens showing no involvement would have developed lesions, had the disease process been allowed to progress until death occurred.

Comparing the proportions of affected organs with results obtained in other filtrate studies revealed several similarities. As shown in Table 3, 100.0 per cent of the livers and 86.7 per cent of the spleens were affected, a finding in close agreement with previous reports. Burmester et al. (1946), Burmester and Cottral (1947), and Burmester and Denington (1947) observed liver involvement in 93, 99, and 98 per cent of affected birds, while splenic lesions appeared in 82, 79, and 83 per cent of the cases, respectively.

Lesions noted in gonads (31.1 per cent) and proventriculus (15.1 per cent) also compared favorably to the earlier results. These reported gonads to be affected in proportions of 31, 22, and 10 per cent, while the proventriculus was involved in nine, three, and zero per cent of examined chicks.

As noted in Table 3, pancreatic involvement occurred in 62.2 per cent of the cases studied and intestinal lesions appeared in 26.7 per cent. These figures were considerably higher than those resulting from inoculation of other filtrable tumor strains. Burmester et al. (1946) observed pancreatic involvement in only nine per cent of affected chicks, and intestinal tumors in four

per cent. The other two reports of cell-free inoculations (Burmester and Cottral, 1947, and Burmester and Denington, 1947) recorded no lesions in either of those organs.

Two tissues which exhibited a considerable degree of involvement were apparently not affected by previous tumor strains studied. These tissues were the lung, of which 44.4 per cent showed gross lesions, and the mesentery, with tumors appearing in 33.3 per cent.

Another deviation from the other three reports was a low incidence of kidney lesions. Only one (2.2 per cent) of the 45 chicks examined revealed a gross lesion in this organ, whereas Burmester et al. (1946), Burmester and Cottral (1947), and Burmester and Denington (1947) observed kidney involvement in about one-half of diseased birds (58, 57, and 50 per cent, respectively). Also, no heart tumors were seen in the 45 inoculates, though they were reported in all three of the earlier papers.

Pathology

Cell type. Neoplastic cells observed in all affected tissues possessed the same characteristic features. Nuclei were large and vesicular, with chromatin appearing at the nuclear membranes, often in clumps (Plate II, Appendix). The majority of nuclei contained a large acidophilic body, assumed to be a nucleolus (Plate II, Appendix). Occasionally two were seen when an oil immersion lens was employed (Plate III, Appendix). The latter two features of the cell were found to be in agreement with a statement by Lucas (1949) which considered an accepted criterion of lymphoid

tumor as the presence of lymphoid cells possessing a clumping of chromatin around enlarged nucleoli. Though some nucleoli were occasionally observed to be centrally located within nuclei, more frequently they appeared in an eccentric position (Plate II, Appendix). Gross et al. (1959) likened these structures to the intranuclear inclusion bodies typical of some viral diseases.

The cytoplasm was slightly basophilic and present in variable amounts, though in the majority of cells it was sparse. In areas of massive proliferation cytoplasm of an individual cell was difficult to discern due to the compact nature of growth (Plate II, Appendix). In addition, cells in these areas exhibited a variety of shapes and sizes, which, according to Gross et al. (1959), would be considered a feature distinguishing the cell as typical of visceral lymphomatosis rather than erythroblastosis. The tumor cells occasionally observed within the vascular system were uniformly round with a distinct cytoplasmic membrane (Plate IV, Appendix). Similarly, Eyestone (1953), in his study of RPL Strain 16, noted that tumor cells were variable in shape when compressed but appeared large and rounded in loose tissues or when seen free at tissue margins.

In all tissues where malignant cells were observed, mitotic figures were frequent (Plate V, Appendix), occasionally as many as 10 to 15 occurring in a single high power field.

For the most part, malignant cells observed in affected tissues were quite similar to those considered by previous authors as typical of visceral lymphomatosis. Gross et al. (1959) most completely described the cell type involved, and stated that it was

generally assumed to be an undifferentiated (stem) cell of the lymphocytic series.

Liver. The most consistent gross features of livers from inoculated chicks were swelling and a variable amount of enlargement (Plates VI, VII, and VIII, Appendix). Swelling was noted to impart a rounded contour to the usually flat hepatic surfaces and to cause a rounding of lobar edges (Plate VIII, Appendix). Though in some cases there were no lesions, liver color was occasionally more reddish than in controls, indicating congestion, and incision of the organ revealed a bulging cut surface which was usually moist with blood. Lesions were quite variable in size and appearance, ranging in number from less than five to more than 20. Most were pin-point foci but areas as large as five mm in diameter were recorded. Occasionally the lesions were raised above the liver surface but generally were not, and their color was not consistent, all shades of yellow, gray, and white being observed. Edges of lesions were generally discrete and the shape usually circumscribed, though exceptions were noted in both cases.

The most characteristic and outstanding feature observed in all microscopic sections was the presence of masses of neoplastic cells (Plate IX, Appendix). Generally, these cellular accumulations appeared in a circumscribed arrangement, but in many cases adjacent nodules formed an irregular pattern of tumorous growth. Such an observation closely resembled microscopic lesions of visceral lymphomatosis as described by Gross et al. (1959), who stated, "the microscopic pattern is usually one of coalescing tumorous foci, which appear to have arisen simultaneously yet independently

of each other".

Nearly all veins in a severely affected liver were completely or partially surrounded by proliferating cells, the latter often forming rounded projections which bulged into the lumina (Plate X, Appendix). Disruption of the endothelium occurred in some cases and tumor cells appeared within the vessel among other blood elements (Plates X and XI, Appendix). This observation could have been an artifact due to tissue sectioning procedure. However, the presence of neoplastic cells in some vessels possessing an intact endothelium supported the probability that such pathology had occurred prior to death of the bird. Arteries and bile ducts were in many cases surrounded by malignant tissue, but this apparently was only a result of their close association with venous structures, as there was no obvious alteration in their histological structure. As stated by Davis and Doyle (1947), a moderate amount of lymphoid infiltration in the chicken liver apparently occurs normally. This tissue was described by them as being perivascular, forming well-defined nodules, and exhibiting no outward diffusion. By means of liver biopsies in several birds, they concluded that outward diffusion from such areas occurred during development of visceral lymphomatosis. Similarly, De Ome (1940) considered lymphomatosis to begin as a mild perivascular hyperplasia of lymphoid elements and felt such lesions were difficult to differentiate from the normal perivascular lymphocytic accumulations. These reports both serve to explain and confirm the characteristic association of malignant tissue with the vascular system, a consistent feature in all liver sections.

Liver cord cells were compressed between and within the tumor cell masses. Though they had undergone considerable atrophy due to pressures exerted by tumor cell growth (Plate XII, Appendix), there was no evidence of nuclear degeneration.

In severely affected livers, it was difficult to determine whether the origin of tumor cells was intravascular or extravascular. However, study of samples taken earlier in the course of the disease gave the impression that lesions were extravascular. In these cases tumor cells appeared as small scattered foci, usually seen in close relation to some part of the venous system. Many of these foci were observed to be separated from blood cells within an adjacent sinusoid by reticuloendothelial cells. It was noted that some isolated tumor cells did occasionally appear among the red blood cells in sinusoids as well as veins, an observation also made by Pentimalli (1941) and Olson (1941) in their descriptions of naturally occurring visceral lymphomatosis. The extravascular location of tumor cells was one of the two characteristics of visceral lymphomatosis cited by Gross et al. (1959) which distinguished it from erythroblastosis.

Spleen. A small proportion of spleens (six of 45) showed no gross alteration. In the others, a predominant feature was enlargement (Plates VI and VII, Appendix), varying from an estimated two to eight times normal size. Regarding the lymphoid lesions, two types of involvement were observed, diffuse and focal. In the former, spleens were extremely pale, a marked contrast to the purple-red color of that organ in control chicks. The focal type was typified by circumscribed, often coalescing, white foci of

various size, the largest measuring six mm diameter (Plate VII, Appendix). There was no obvious capsular thickening but this structure was so tense that upon incision parenchyma bulged from beneath it. The splenic tissue itself was extremely pulpy, particularly in organs showing the greatest degree of enlargement.

The most striking microscopic feature in splenic tissues was a disorganization of the normal architecture. In sections from normal chicks, lymphoid tissue appeared as circumscribed foci scattered throughout the red pulp. Affected spleens, however,. exhibited large irregularly shaped masses of tumor cells which had replaced normal lymphoid follicles. Due to proliferation of this tissue there was a subsequent condensation of cellular elements normally comprising the red pulp areas. Thus there appeared microscopically to be a severe congestion in the latter, though in gross examinations this was not noted. In addition, condensation of the reticular cells gave red pulp areas a marked eosinophilic reaction which was particularly noticeable at low power magnifications. As in other affected tissues, mitotic figures were numerous in affected spleens. However, normal spleens similarly displayed a considerable degree of proliferative activity, as would be expected considering their hematopoietic function. The marked difference between normal lymphoid follicles in control spleens and the neoplastic masses of tumorous spleens was the cell type predominant in each. Tumor cells were typically irregular in shape, exhibited clumping and margination of nuclear chromatin, and contained large acidophilic nucleoli. These were easily differentiated from cells of the lymphoid series which were present in control spleens.

Such cells were generally smaller, more uniform in size, the nuclear chromatin was homogeneously distributed, and no nucleoli were noted.

Pancreas. Minimum pancreatic involvement occurred in the form of numerous, circumscribed, gray lesions which presented an opaque glassy appearance. They ranged in size from pin-point foci up to one mm in diameter, were not raised above the surface, and exhibited entire edges. In more severely affected organs lesions were similar in color and character, but much larger. Some were gray masses up to four mm in diameter and these bulged above the surface of adjacent normal tissue.

Microscopically, there was no consistency in location of cell masses, some appearing at the periphery of the organ and others internally. In either case, lesions consisted of neoplastic cells and some atrophied acinar cells. There was no evidence of necrotic processes or vascular changes. Malignant tissue extended from most of the tumorous masses into the surrounding pancreatic parenchyma by numerous finger-like projections.

Lung. The color of lung tumors was usually gray or gray-white and they varied in size from less than one mm in diameter to areas involving up to one-half the entire organ. Where the smaller foci appeared, as many as 10 were recorded in a single lung. Some of the larger lesions exhibited a yellow caseous material in the centers. A majority of tumors were discovered on costal surfaces, though some were observed on pleural surfaces. In the first location they were raised whereas in the second case they were not. All were circumscribed and presented smooth edges. Frequently,

though no lesions were seen on initial examination of a lung, incision of the organ revealed a thick, gray tissue surrounding the primary bronchus.

Histologically, tumor tissue was generally well-defined, though never encapsulated. Each lesion consisted of a tumor cell mass within which appeared remnants of bronchi and parabronchi (Plate XIII, Appendix). The lumina of these structures were in some cases filled with a serofibrinous exudate occasionally containing red blood cells and tumor cells. Blood vessels were surrounded by the proliferating malignant tissue, but remained intact, and tumor cells were seen both in veins and arteries. The largest lesions, in which a yellow caseous material was observed grossly, exhibited necrotic areas. Hemorrhage, serofibrinous exudate, and karyorrhexis of nuclei were the predominant changes noted.

Mesentery. The location of mesenteric neoplasms was consistently observed to be associated with the major blood vessel of that structure. Tumorous tissue masses were solid, gray to white in color, and in most cases so extensive as to completely surround the vessel. The largest recorded mass was eight mm long, six mm wide, and four mm thick. A few large lesions of this type displayed foci of yellow caseous material, assumed to be necrotic tissue.

Microscopically, tumorous tissue of mesenteries exhibited a greater degree of anaplasia than was seen in any other tissue examined. Tumor cells in some cases invaded and at many points completely obliterated areas of the vascular walls, some cells also

appearing within the lumina. In the majority of lesions tissue masses consisted only of tumor cells. Occasionally, however, these tended to appear as clumps within a loose stroma of reticular type tissue. In such an area there were often a surprising number of spindle-shaped cells, giving the tumor a fibrosarcomatous appearance. Caseation necrosis was the pathology responsible for yellow foci observed grossly in some tumor masses.

Ovary. In all cases of ovarian involvement, the affected organ was enlarged to some degree, the largest estimated to be four times normal size. Color of the tumorous organs was invariably chalk-white as compared to a gray transparent appearance observed in ovaries from controls (Plate XIV, Appendix).

Microscopic sections revealed that neoplastic involvement was of a diffuse nature. Tumor cells were scattered throughout the organ's reticular stroma with no recognizable pattern of growth, and most follicles were completely replaced by tumor tissue. The few which remained appeared at the periphery and some of these were being invaded by proliferating malignant cells. This invasion was accomplished by a disruption of follicular basement membranes and subsequent replacement of follicular cells by the large tumor cells. Accumulations of lymphoid cells were occasionally observed in normal ovarian tissue, but these were small mature cells, bearing little similarity to those seen in tumorous tissues.

Testicle. In male chicks with involvement of the gonads, only one testicle was usually affected, and enlargement was the principal manifestation of pathology. The swelling was variable in degree, but the most enlarged testicles were estimated to be

three times normal size. A color change from the normal yellow to a grayish opaque appearance occurred in the most severely affected organs.

Histologically, lesions of the testicles were usually not well defined. The typical picture was a blending of tumor tissue with areas of normal testicular structure, remnants of seminiferous tubules as well as reticular stroma appearing among neoplastic cells. Some tubules showed destruction of basement membranes and cellular invasion while others were not altered.

Intestine. Grossly, the most frequent location of intestinal tumors was the duodenum, where they almost always appeared in conjunction with an adjacent pancreatic lesion. Neoplastic lesions of the two organs were often fused to form one mass of tumor tissue, the largest recorded being five mm in diameter. These lesions were gray to white in color, occasionally containing yellow foci in the central portions. The tumors generally appeared as circumscribed, elevated nodules, though some were merely diffuse areas of discoloration. Tumors in other parts of the intestinal tract presented a similar character to those of the duodenum with the exception that they were usually smaller, one to two mm in diameter. The greatest number of intestinal tumors observed in a single bird was three.

In microscopic studies of the intestine, regardless of the portion affected, histopathology was similar. Malignant tissue was well localized and usually involved the entire wall from serosa to mucosa. Cellular infiltration of the tunica muscularis caused separation of muscle fibers and completely replaced them

in many areas. A few small foci of coagulation necrosis were the only degenerative effects seen and these were not present in all lesions. When tumor tissue extended through the muscular tunics, proliferating cells appeared as a solid mass containing scattered elements of the mucosal epithelium. In some of the largest masses tips of the villi were ulcerated and necrotic tissue had sloughed into the intestinal lumina.

Proventriculus. Examination of the proventriculus in each of the 45 chicks revealed no gross pathology on serosal surfaces, all recorded lesions being discovered following incision of the organ. Tumors appeared on mucosal surfaces as circumscribed white nodules ranging in size from one to five mm in diameter. Rarely was more than one lesion observed per organ.

A histological review of the proventriculus by Trautmann and Piebiger (1952) described the presence of large lymphocytic aggregations between superficial glands in the propria. Study of control and affected organs revealed such cellular accumulations in that location. In tumorous tissues, however, the number of cells was greatly increased and they were of the type previously described as characterizing tumors resulting from injection of the filtrable agent.

Kidney. The only kidney tumor found in gross examinations was a small, circumscribed, white area measuring one mm in diameter, and was located on the surface of the left anterior lobe. In many chicks kidneys appeared paler than those of controls but this was not considered significant since affected birds usually exhibited a generalized tissue anemia.

Considering the low incidence of grossly visible lesions, an unexpected observation in histopathology studies was the frequency of renal infiltration by tumor cells. This was probably due to the diffuse nature of lesions. Malignant cells, though concentrated within an area, were dispersed between renal tubules, pushing those structures apart. In a few cases, proliferating cells formed foci of tumor tissue, but the latter were definitely of microscopic size. The only noted effect on glomeruli and tubular epithelium was atrophy and this was not frequently seen. Cellular accumulations appeared in both cortical and medullary regions and there appeared to be no predilection for either site. All affected kidneys revealed the presence of tumor cells within the vascular bed.

Adrenal. Gross lesions were not observed in adrenal glands, but due to the small size of this organ, little could have been noted except an enlargement.

Histopathology examinations revealed neoplastic growth in a very few glands, and these were not extensive. In such cases, malignant tissue tended to form a band at the periphery of the organ, sending projections into central portions of the parenchyma. These followed the course of blood vessels between cord cells in a pattern similar to that of chromaffin cell distribution. Chromaffin cells, however, were easily distinguished from tumor cells by their more abundant, less basophilic cytoplasm and pale nuclei which lacked nucleoli.

Heart. Though tumors of the heart were not seen grossly, a very few heart sections did show minimal degrees of cell invasion

occurring between the muscle fibers. Separation and isolation of fibers were observed but there was no evidence of tissue destruction.

Thymus. Necropsies and the gross weight survey of affected chicks revealed that thymus glands had undergone marked atrophy. Lobes of the organ in control chicks were plump, oval structures, grayish in color, and were easily removed. In inoculates, the lobes were smaller, flatter, and appeared ivory in color, making their separation from surrounding fatty tissue very difficult.

Tissue sections of the normal avian thymus revealed that the cortex comprised about one-half to two-thirds of the gland. This tissue consisted of cells which were characterized by small nuclei, homogeneous chromatin distribution, absence of nucleoli, and very scant cytoplasm. According to Danschakoff (1916) such cells were considered to be derivatives of small lymphocytes which in turn had originated from mesenchyme cells.

One of the two outstanding microscopic alterations observed in thymus sections from inoculates was extreme depletion of cortical portions. In most sections depletion was so extensive that only a few cells like those of the normal thymus cortex were found. There were no pathological changes observed to definitely indicate what had become of the cells, but notation of several apparently pyknotic nuclei suggested the possibility that some might have undergone degeneration.

The second pathological manifestation of the injected agent, typical of that cited in all other affected tissues, was a proliferation of large undifferentiated tumor cells. As previously

reported in this paper, the malignant cell of visceral lymphomatosis has been assumed to be a stem cell of the lymphocyte (Gross et al., 1959). Considering lymphocyte formation as an activity of the thymus, it was thought that the proliferating cells were possibly those which normally would have become lymphoblasts. Lucas and Jamroz (1961), describing lymphocytogenesis in the avian thymus, reported that precursors for the lymphocyte line were reticular cells. The latter were typically observed to possess well-define nucleoli which were lost upon transformation to lymphoblasts. This observation, however, was made with smear preparations, and the authors pointed out that sections of the organ might demonstrate the presence of a nucleolus in immature stages of lymphocytic development. Therefore, available knowledge indicated that the tumor cells observed in this study, which consistently possessed a large nucleolus, could be classified as either reticular cells or lymphoblasts.

CONCLUSIONS

Filtrates prepared from tissues containing the visceral leukosis-like agent and inoculated into susceptible chicks reproduced the disease in a high percentage of birds. Mortality rates were similar to those incurred by filtrates of previously reported tumor strains, but course of the disease was much shorter. Therefore, as a potential research tool for study of malignancy, the agent characterized by this report was considered to present a distinct advantage over other tumor strains, since rapid development of lesions allows an early evaluation of experimental results

and greatly facilitates design of further tests. In addition, the extensive degree of tumor cell proliferation which was observed microscopically suggested that this agent could be of great value in studying metabolic activity and similar phenomena of neoplastic cells.

In these studies sterility checks were conducted on all filtrate preparations and filters were repeatedly tested for bacterial retention. Results of these procedures indicated that the infective filtrates were cell-free and that the agent present was filtrable through bacteria-retaining filters.

Based on necropsy observations and gross weights, the liver and spleen were found to be the organs most frequently affected by the carcinogenic agent. This finding concurred with previous reports of other tumor strains and of naturally occurring cases. A relatively high incidence of pancreatic, lung, and mesenteric lesions, and the absence of heart or kidney involvement in inoculates were concluded to be unique features of the disease. Gross and microscopic examinations revealed neoplasia in a large number of tissues and this was thought to reflect the multicentric activity of a rapidly multiplying agent present in infective filtrates.

The notation of thymus atrophy in all inoculated chicks was concluded to be a manifestation of the condition resulting from inoculation. It was not determined, however, whether this atrophy was a primary glandular response to the filtered agent or a lesion secondary to interference with some vital body function.

Microscopic appearance of tumor cells and organ histopathology were in close agreement with literature descriptions of

experimental and naturally occurring visceral lymphomatosis cases. This finding was assumed to support a classification of the disease studied as leukosis, or at least leukosis-like.

SUMMARY

Successful transmission of an avian lymphoid tumor by inoculation of chicks with cell-free preparations was accomplished. A comparison with controls indicated that in inoculated chicks the body and thymus weights were decreased, while liver and spleen weights were markedly increased. Gross and microscopic lesions in involved tissues were summarized and compared to descriptions of previous writers, a striking similarity being noted in most cases. The outstanding and consistent pathology observed was a massive proliferation of neoplastic tissue replacing normal structures, and on the basis of microscopic examination, malignant cells appeared to be of a lymphoid nature, though highly undifferentiated.

ACKNOWLEDGEMENTS

The author wishes to express her appreciation to Dr. M. J. Twiehaus, head of the Department of Pathology, for his guidance during completion of this study and preparation of the thesis. The advice and encouragement of Dr. H. C. Mussman is also gratefully acknowledged.

Special thanks are offered to the writer's husband, Lyle D. Miller, for his assistance in many phases of the work.

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APPENDIX

PREPARATION OF SIMMS SOLUTION (Simms and Sanders, 1942)

Mother Solution I:

NaCl	80.00 gm
KCl.	2.00 gm
$CaCl_2 \cdot 2HOH$	1.47 gm
$MgCl_2 \cdot 6HOH$	2.03 gm
Distilled water.	500.00 cc

Autoclaved.

Mother Solution II:

$NaHCO_3$	10.10 gm
Na_2HPO_4.	2.13 gm
Phenol Red	0.10 gm
Distilled water.	400.00 cc

Autoclaved.

Dextrose Solution:

Dextrose	10.00 gm
Distilled water.	100.00 cc

Autoclaved.

With sterile technique, the dextrose solution was added to Mother Solution II. Mother Solutions I and II were stoppered and stored in a refrigerator.

Working Solution:

Mother Solution I. . . .	25.00 cc
Distilled water. . . .	450.00 cc

Autoclaved. When cool, the following was added by sterile technique:

Mother Solution II . . .	25.00 cc

The working solution was stored in a refrigerator and kept at a pH of about 7.4, or an orange-red color. Adjustment of the pH was made by bubbling 5 per cent carbon dioxide or oxygen through the solution.

Table 4. Body, liver, spleen, and thymus weights of 30 control chicks.

Bird Number	Body weight gm	Liver gm	Liver per cent body weight	Spleen gm	Spleen per cent body weight	Thymus gm	Thymus per cent body weight
1	125.0	4.32	3.46	0.143	0.114	0.847	0.678
2	149.0	5.44	3.65	0.155	0.104	0.704	0.473
3	91.4	3.67	4.02	0.089	0.097	0.345	0.529
4	119.0	4.88	4.10	0.167	0.140	0.720	0.605
5	108.0	3.86	3.57	0.139	0.129	0.437	0.405
6	94.5	3.49	3.69	0.094	0.099	0.297	0.314
7	89.0	3.21	3.61	0.093	0.104	0.314	0.353
8	102.0	3.28	3.22	0.099	0.097	0.376	0.369
9	137.0	4.46	3.26	0.113	0.083	0.793	0.579
10	126.0	4.31	3.42	0.099	0.079	0.376	0.298
11	91.7	3.98	4.34	0.087	0.095	0.434	0.473
12	108.0	3.86	3.57	0.153	0.142	0.413	0.382
13	110.0	4.15	3.77	0.081	0.074	0.756	0.687
14	99.3	4.04	4.07	0.097	0.098	0.276	0.278
15	152.0	5.60	5.49	0.246	0.162	0.697	0.459
16	133.0	7.61	5.72	0.253	0.190	0.435	0.327
17	142.0	4.49	3.16	0.199	0.140	0.476	0.335
18	153.0	5.42	3.54	0.280	0.183	0.732	0.478
19	113.0	3.96	3.50	0.158	0.140	0.307	0.272
20	132.0	4.71	3.57	0.123	0.093	1.090	0.825
21	161.0	7.22	4.48	0.258	0.160	0.755	0.469
22	160.0	5.29	3.31	0.143	0.089	0.823	0.514
23	134.0	4.93	3.68	0.225	0.168	0.217	0.162
24	126.0	5.00	3.97	0.220	0.175	0.552	0.438
25	130.0	5.98	4.60	0.160	0.123	0.394	0.303
26	121.0	5.14	4.25	0.236	0.195	0.579	0.479
27	128.0	5.20	4.06	0.141	0.110	0.581	0.454
28	113.0	4.72	4.18	0.116	0.103	0.675	0.597
29	104.0	4.27	4.11	0.125	0.120	0.344	0.331
30	104.0	3.44	3.31	0.110	0.106	0.335	0.322
Average	121.9	4.66	3.89	0.157	0.124	0.535	0.440

Table 5. Body, liver, spleen, and thymus weights of 45 filtrate-inoculated chicks.

Bird Number	Body weight gm	Liver gm	Liver per cent body weight	Spleen gm	Spleen per cent body weight	Thymus gm	Thymus per cent body weight
1060	71.1	5.22	7.34	0.317	0.446	0.128	0.180
1055	86.3	5.96	6.91	0.227	0.263	0.128	0.148
1044	73.2	4.70	6.42	0.266	0.363	0.119	0.163
1047	71.5	4.39	6.14	0.138	0.193	0.130	0.183
1057	73.5	4.44	6.04	0.103	0.140	0.143	0.195
1046	78.4	5.80	7.40	0.442	0.564	0.221	0.282
1053	78.0	5.00	6.41	0.117	0.150	0.284	0.364
1064	73.0	4.89	6.70	0.102	0.140	0.094	0.129
1049	73.4	4.00	5.45	0.090	0.123	0.053	0.072
1065	80.6	5.64	7.00	0.190	0.236	0.122	0.151
1059	84.4	7.74	9.17	0.442	0.524	0.161	0.191
1069	84.9	6.26	7.37	0.356	0.419	0.173	0.204
1056	81.1	6.88	8.48	0.409	0.504	0.108	0.133
1050	82.5	7.75	9.39	0.878	1.064	0.132	0.160
1058	78.9	5.98	7.58	0.339	0.430	0.301	0.381
1048	82.4	4.36	5.29	0.218	0.265	0.247	0.300
1041	76.6	5.39	7.04	0.257	0.336	0.071	0.093
1248	69.1	4.56	6.60	0.386	0.559	0.110	0.159
1245	72.8	5.94	8.16	0.458	0.629	0.112	0.154
1261	75.3	5.81	7.72	0.444	0.590	0.114	0.151
1253	69.4	4.15	5.98	0.450	0.648	0.105	0.151
1251	109.0	7.44	6.83	0.775	0.711	0.277	0.254
1242	80.2	5.82	7.26	0.458	0.571	0.041	0.051
1239	60.7	4.36	7.18	0.134	0.221	0.043	0.071
1246	83.1	6.59	7.93	1.640	1.974	0.122	0.147
1252	101.0	9.32	9.23	1.780	1.762	0.290	0.287
1265	93.1	9.53	10.20	0.992	1.066	0.202	0.217
1307	70.9	5.66	7.98	0.353	0.498	0.112	0.158
1321	72.9	5.42	7.44	0.423	0.580	0.095	0.130
1319	76.2	5.23	6.86	0.176	0.231	0.108	0.142
1299	79.9	5.79	7.25	0.473	0.592	0.110	0.138
1296	64.4	4.74	7.36	0.397	0.617	0.100	0.155

Table 5 (concl.).

Bird Number	Body weight gm	Liver gm	Liver per cent body weight	Spleen gm	Spleen per cent body weight	Thymus gm	Thymus per cent body weight
1313	69.6	5.11	7.34	0.217	0.312	0.116	0.167
1298	73.3	5.23	7.14	0.438	0.598	0.109	0.149
1300	75.0	5.47	7.29	0.303	0.404	0.138	0.184
1303	81.1	5.92	7.30	0.201	0.247	0.116	0.143
1297	78.2	5.70	7.29	0.202	0.258	0.157	0.201
1760	79.8	6.85	8.58	0.447	0.560	0.135	0.169
1764	76.8	6.28	8.18	0.387	0.503	0.121	0.158
1762	76.9	6.07	7.89	0.264	0.343	0.096	0.125
1761	73.3	5.38	7.34	0.344	0.469	0.144	0.196
1759	82.9	6.88	8.30	0.449	0.542	0.143	0.172
1754	72.8	5.26	7.23	0.163	0.224	0.061	0.084
1750	77.9	7.22	7.27	0.563	0.723	0.205	0.263
1758	87.6	7.28	8.31	0.259	0.296	0.111	0.127
Average	78.1	5.85	7.41	0.410	0.509	0.138	0.174

PLATE I

PLATE II

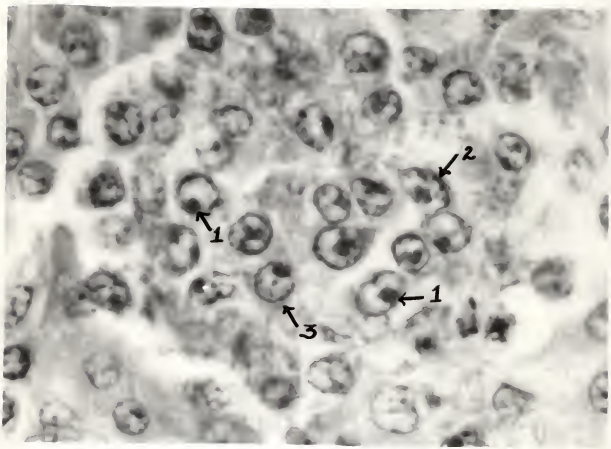


PLATE III

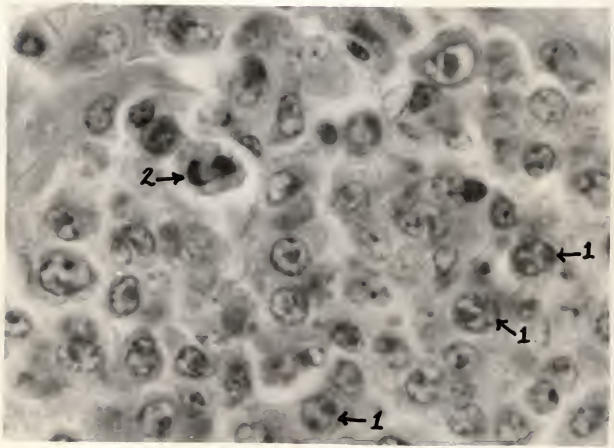
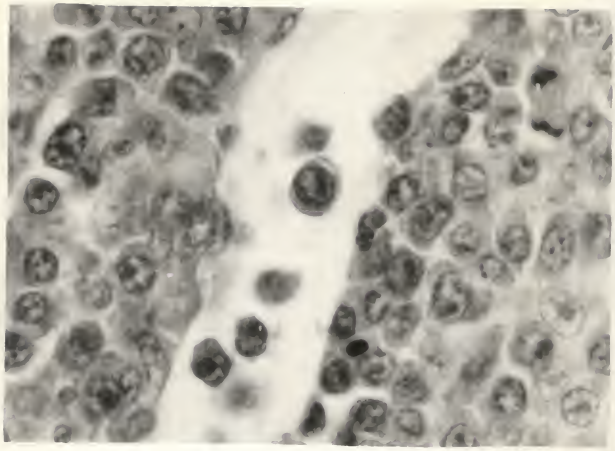


PLATE IV



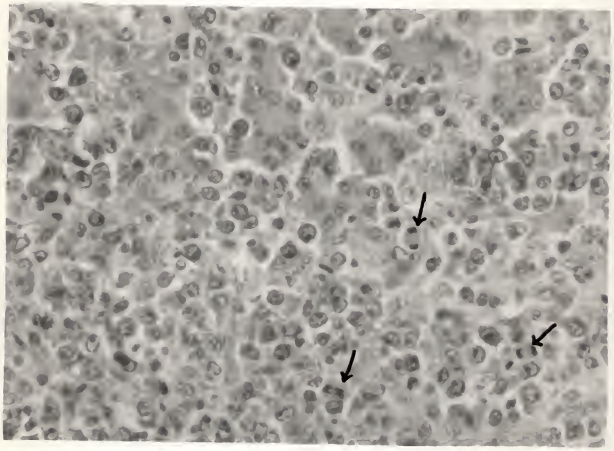
EXPLANATION OF PLATE IV

Lymphoid cells within the lumen of a hepatic vein, all exhibiting distinct cytoplasmic membranes. (970x).

EXPLANATION OF PLATE V

Tumor cell proliferation in liver section. Note mitotic figures (arrows). (450x).

PLATE V



EXPLANATION OF PLATE VI

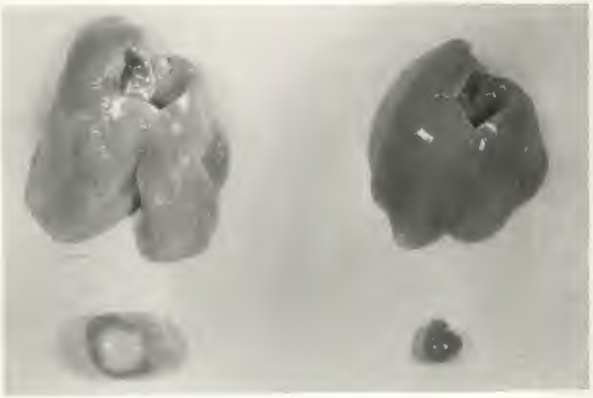
Livers and spleens of four inoculates showing variable degrees of gross lymphoid involvement. Control liver and spleen at right. Eleventh day.

PLATE VI

EXPLANATION OF PLATE VII

Enlargement and focal lesions in liver and spleen of inoculate with normal organs appearing at right. Thirteenth day.

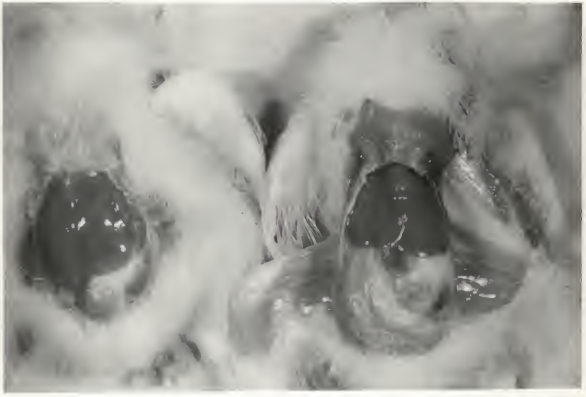
PLATE VII



EXPLANATION OF PLATE VIII

Affected chick at left depicts enlargement and swelling of the liver 11 days after inoculation. Control chick of same age appears at right.

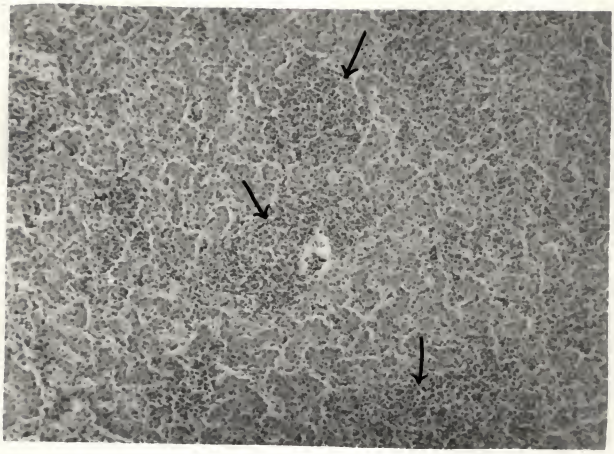
PLATE VIII



EXPLANATION OF PLATE IX

Liver section of inoculate illustrating accumulations of tumor cells (arrows). (100x).

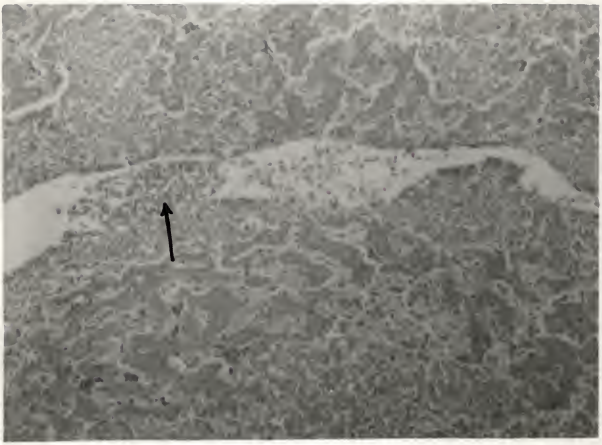
PLATE IX



EXPLANATION OF PLATE X

Liver section showing perivascular neoplastic tissue with tumor cells penetrating vessel wall and appearing within the lumen (arrow). (100x).

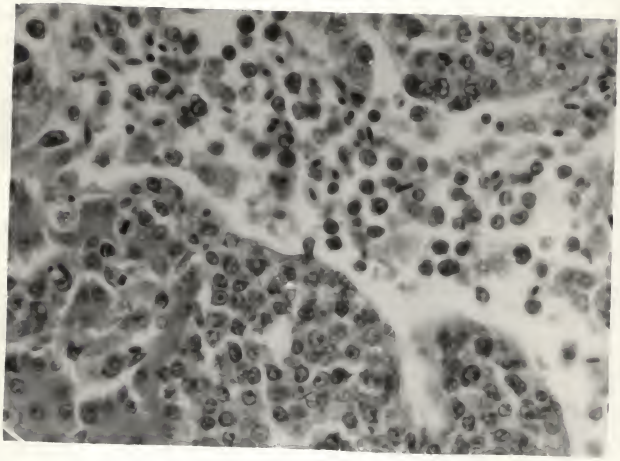
PLATE X



EXPLANATION OF PLATE XI

Hepatic vein containing numerous tumor cells. (450x).

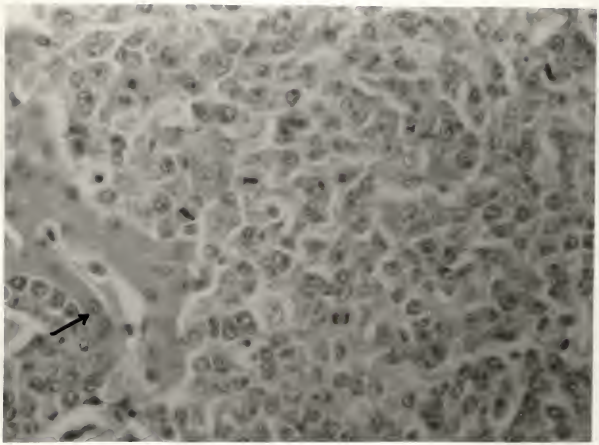
PLATE XI



EXPLANATION OF PLATE XII

Neoplastic cells in liver producing atrophy of hepatic cord cells (arrow). (450x).

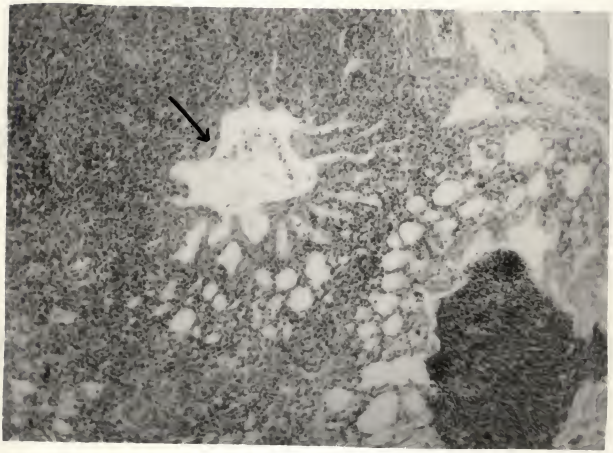
PLATE XII



EXPLANATION OF PLATE XIII

Section of lung from inoculate depicting tumorous growth surrounding a parabronchus (arrow). (100x).

PLATE XIII



EXPLANATION OF PLATE XIV

Neoplastic ovary of inoculate at right showing chalk-white color and extreme enlargement as compared to that of control on the left. Thirteenth day.

PLATE XIV

GROSS AND MICROSCOPIC LESIONS IN CHICKS INOCULATED WITH A FILTRATE OF AN AVIAN VISCERAL LEUKOSIS-LIKE AGENT

by

JANICE M. LILLY

B. S., Kansas State University, 1960
D. V. M., Kansas State University, 1962

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1963

Since 1946 several reports involving successful transmission of avian visceral lymphomatosis with cell-free filtrates have appeared in the literature, many of these studies utilizing laboratory-propagated tumor strains. A highly virulent lymphoid tumor isolated at the Kansas State University Department of Pathology in 1957 has been maintained in fowl by 182 serial passages of cellular inocula. It was the object of this investigation to confirm transmissibility of that tumor strain by cell-free filtrates and to characterize the agent's pathological effects on inoculated chicks.

A total of 255 chicks four to eight days of age were inoculated in 10 experiments. Livers and spleens of donor birds were collected and tissue homogenates prepared. The suspensions were then filtered through #02 Selas candle filters to obtain cell-free inocula.

Tissues most frequently affected by the filtrable agent were determined in 45 inoculated chicks by means of gross and microscopic examinations. The body, liver, spleen, and thymus of each bird were weighed and similar procedures were repeated on each of 30 control chicks.

Transmission of the disease by filtrates was observed in all 10 trials. Mortality rates of experiments ranged from 70.0 to 90.0 per cent, with deaths occurring in 80.4 per cent of the 255 chicks inoculated. Mean death times in the various tests ranged from eight to 17.0 days and the average days-to-death for all chicks was 11.1.

Necropsy of 45 filtrate-inoculated chicks revealed that gross lesions of the viscera, in decreasing order of frequency, appeared as follows: liver, spleen, pancreas, lung, mesentery, gonads, intestine, proventriculus, and kidney.

Body weights of inoculates were markedly lower than those of controls, affected livers exhibited a two-fold enlargement, and spleens were generally enlarged to four times normal. On the basis of per cent body weight, thymus glands of affected birds were approximately one-half normal size.

The outstanding and consistent histopathology in all affected tissues was an extensive invasion by masses of large, undifferentiated, neoplastic cells.

Results of the study were concluded to indicate the following: a filtrable agent of the leukosis-like disease was capable of reproducing the condition in a high per cent of inoculated chicks; deaths occurred more rapidly than those incurred by any previously reported tumor strains; gross lesions appeared in many body tissues, but livers and spleens were most frequently involved, accompanied by an atrophy of thymus glands; histopathology was markedly similar to that of experimental and naturally occurring visceral lymphomatosis as described by most writers.